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(54) [TITLE OF THE INVENTION] Fish and Shellfish Culturing Method

(57) [ABSTRACT]

[Constitution] The culturing method of the present invention consists of raising fish and shellfish by adding a feed incorporating live lactobacilli to a body of water in which fish and shellfish are cultured.

[Effect] According to the culturing method of the present invention, diseases of cultured fish and shellfish are prevented, fish and shellfish growth is promoted, and the method is also superior in terms of safety.

[CLAIMS]

1. A method for culturing fish and shellfish comprising: raising fish and shellfish by adding feed and live lactobacilli to a body of water in which fish and shellfish are cultured.

2. The method for culturing fish and shellfish according to claim 1, wherein the added amount of lactobacilli is 10^6 or more organisms per 1 g of feed.

[DETAILED DESCRIPTION OF THE INVENTION]

[0001]

[Field of Industrial Application]

The present invention relates to a method for culturing fish and shellfish such as shrimp, halibut, blowfish or eel. According to the culturing method of the present invention, the occurrence of disease in fish and shellfish is prevented and the growth of fish and shellfish is promoted.

[0002]

[Prior Art and Problems to be Solved]

The need for fisheries industries, including culturing, is becoming increasingly great from the viewpoint of protecting marine resources and in terms of international politics. The fisheries industry manages and artificially breeds marine products such as fish and shellfish using facilities such as rafts, ledges, longlines, underwater fish preserves and ponds.

[0003]

High-density culturing is considered to be preferable for economic reasons when culturing fish and shellfish in this manner, and blended feeds having a high protein content are widely used for feed instead of live feed. Consequently, large amounts of residual feed and excrement accumulate on the sea bottom in the form of sludge, resulting in contamination of the seawater and bottom, and causing seasonal outbreaks of diseases among cultured fish and shellfish due to bacterial growth.

[0004]

Although antibiotics or live microbial agents are ordinarily used in veterinary medicines, in the case of using antibiotics, there are numerous problems such as the appearance of resistant strains and limitations on use prior to shipment. Consequently, the production yield of cultured fish and shellfish is not that high, being on the order of 40% to 60% in the case of tiger prawns, for example.

[0005]

In addition, although live microbial agents are widely used as veterinary medicines in the field of animal husbandry, they have not been used in the field of marine products. Although conventional examples of live microbial agents include photosynthetic bacteria (PSB), microbial mixtures (Toaraze: Toa Pharmaceutical) and *Bacillus cerius toyoi* (Toyoserine, Toyo Jozo), in the case of culturing facilities having a poor environment, these live organisms are unable to survive, use under optimum conditions is difficult, or contribute to increased costs, thereby preventing them from being used practically.

[0006]

An object of the present invention is to provide a culturing method capable of preventing disease in cultured fish and shellfish while also having superior safety.

[0007]

[Means for Solving the Problems]

As a result of conducting various extensive studies based on the circumstances described above, the inventors of the present invention found that by culturing fish and shellfish using a feed containing live lactobacilli, the occurrence of diseases in the fish and shellfish can be inhibited, thereby leading to completion of the present invention.

[0008]

Namely, the present invention provides a fish and shellfish culturing method comprising raising fish and shellfish by adding feed and live lactobacilli to a body of water in which fish and shellfish are cultured. In the culturing method of the present invention, both conventional commercially available blended feed and homemade feed used as feed for fish and shellfish can be used.

[0009]

Although any known lactobacilli can be used for the live lactobacilli used in the method of the present invention, *Enterococcus faecium* is preferable, and *Enterococcus faecium* SHO-31 (Fermentation Research Institute Accession No. 12253) is particularly preferable. This microorganisms are able to survive in seawater and sludge, and survive by colonizing the intestines of fish and shellfish. In addition, they also inhibit the proliferation of bacteria in residual feed and excrement, and are highly safe for fish, shellfish and humans.

[0010]

These lactobacilli are preferably added to feed. There are no particular limitations on the added amount of lactobacilli to feed, and is preferably added at 10^6 or more organisms per 1 g of feed. If the amount of lactobacilli is less than this amount, adequate disease preventive effects are unable to be obtained.

[0011]

When adding the lactobacilli to the feed, frozen lactobacilli equivalent to 0.1 to 2% of the weight of the dry feed are suspended in water in an amount equivalent to about 10 to 50% of the weight of the dry feed, and the resulting suspension is uniformly sprayed onto the feed.

[0012]

The addition of lactobacilli to blended feed (dry pellets) is carried out in the manner described below. In the case of adding to fine particulate feed, a predetermined amount of thawed lactobacilli is dissolved in water equivalent to 50% of the amount of feed to prepare diluted lactobacilli. These diluted lactobacilli are then sprayed onto the fine particulate feed followed by mixing well. In addition, in the case of adding to adult shrimp feed, a predetermined amount of thawed lactobacilli is dissolved in water equivalent to 10% of the amount of feed to prepare diluted lactobacilli. These diluted lactobacilli are then uniformly sprayed onto the feed followed by mixing well.

[0013]

Fish and shellfish are cultured by normally giving this feed about one to three times per day in the same manner as conventional feed.

[0014]

As a result of using the feed of the present invention for fish and shellfish, effects are obtained including prevention of disease, therapeutic effects, promotion of growth and improvement of feeding efficiency.

[0015]

Furthermore, the lactobacilli used in the method of the present invention are preferably lactobacilli that have been pure-cultured and concentrated so that the feed contains 10^{10} organisms/mL or more. The use of such lactobacilli allows the lactobacilli to act effectively within the bodies of the fish and shellfish.

[0016]

In addition, lactobacilli also have a function that inhibits the proliferation of *Vibrio cholerae*, and is believed to inhibit the proliferation of bacteria in residual feed and excrement. Namely, when lactobacilli (SHO-31) and *Vibrio cholerae* (Vibrio strain PJ) were dual-cultured in a Petri dish (using BHI-HNG medium), *Vibrio cholerae* was determined to not proliferate. In this manner, lactobacilli have a growth inhibitory function on *Vibrio cholerae*.

[0017]

In addition, lactobacilli contain numerous components and metabolites effective for the growth of fish and shellfish. They contain particularly high contents of proteins and amino acids, while also containing physiologically active substances such as nucleic acids and enzymes. The contents of these components are compared with other typical feeds and are shown in Table 1.

[0018]

Table 1

Sample	Crude protein	Crude fat	Crude fiber	Ash	Soluble sugars
Lactobacillus SHO-31	62.00	ND	1.17	10.51	26.32
Photosynthetic bacteria*	57.95	7.91	2.92	4.40	20.83
Chlorella*	53.76	6.31	10.33	1.52	19.28
Soybean*	38.99	19.33	5.11	5.68	30.93
Rice*	7.48	0.94	0.35	0.72	90.60

Values indicate %/dry weight (g)
* Process Biochemistry, 13, 9, 27-30

[0019]

[Test 1]

When tiger prawns were cultured under the conditions indicated below, feeding efficiency was found to improve as shown in Table 2, and growth promotion of the tiger prawns was remarkable. Since tiger prawns are characterized by diving to sea bottom and are cannibalistic, there is a high likelihood of their being infected by pathogens in sludge, and the cause of death of cultured tiger prawns is almost always of a bacterial nature.

[0020]

Table 2

Test Parameter	Test group A	Test group B	Control group
Total feed consumption (g)	28.4	28.7	28.7
Initial mean body weight (g)	0.90	0.90	0.93
Final mean body weight (g)	1.30	1.24	1.22
Mean body weight gain (%)	44.44	37.78	31.18
Initial gross body weight (g)	18.86	18.66	19.53
Final gross body weight (g)	26.02	25.90	24.37
Gross body weight gain (%)	37.96	37.33	24.78
Feeding efficiency (%)	42.02	40.88	28.11
Shedding rate (%)	185.00	145.0	165.0
Survival rate (%)	95	100	95

Test group (A): Addition of lactobacilli and other microbial mixture to feed
Test group (B): Addition of lactobacilli only

[0021]

Test Conditions:

Feed: Lactobacilli were added at the time of feed preparation followed by molding into moist pellets with the feed raw materials

Test tank: 30 L

Water temperature: 23°C

No. of tiger prawns: 21 per group

Test period: 18 days

[0022]

[Test 2]

Enterococcus faecium is salt-resistant microorganism that demonstrates a particularly high level of survivability in seawater, and demonstrates a high survival rate even in seawater and sludge subjected to harsh environmental conditions. Lactobacilli were added to seawater located above the sludge at a tiger prawn farm followed by an investigation of changes in the lactobacilli count and total bacterial count present in the sludge over time. Those results are shown in Table 3.

[0023]

Table 3

	Bacterial count (cfu/mL)		Lactobacilli count
Testing time	Lactobacilli count	Total bacterial count	Total bacterial count
Start of test	3.2×10^7	6.1×10^6	5.2
24th day	1.6×10^7	3.5×10^6	4.6
70th day	1.7×10^5	4.2×10^4	4.0

Test Conditions:

Test tank: Natural seawater 30 L

Amount of sludge at tiger prawn farm: 30 L

Water temperature: Approx. 23°C

Amount of lactobacilli added: Adjusted to a concentration of 3.2×10^7 cfu/mL in the seawater at the start of the test

Test sample: Diatoms growing on the sludge were primarily sampled and used to measure bacterial count.

[0024]

[Test 3]

Since the lactobacilli used in the present invention are derived from the body and similar types of lactobacilli are present in abundance in the natural world, they are highly safe as feed additives for fish and shellfish while also being safe for humans.

[0025]

Lactobacilli were added at a high concentration to seawater, and 100 tiger prawns in the nauplius and zoea stages, during which they are susceptible to the ambient environment, were added while fasting followed by observation of their growth. As a result, the lactobacilli addition group demonstrated a higher survival rate than the control group as shown in Table 4, indicating that the presence of lactobacilli is highly safe with respect to shrimp larvae.

[0026]

Table 4

	Age			
Test group	1 day	2 days	3 days	4 days
Lactobacilli addition group	100	85	82	250
Control group	100	100	100	100

Values indicate the survival rates (%) of the test group
based on a value of 100% for the control group.

Test Conditions:

Test group: Addition of lactobacilli at 1×10^8 organisms/mL

Control group: Lactobacilli not added

[0027]

[Test 4]

Culturing fish and shellfish according to the method of the present invention results in a high yield and superior feeding efficiency as shown in Table 5.

Evaluation Conditions:

Breeding site: Concrete tanks located in breeding houses

No. of breeding houses: 4

Tank surface area: 600 m^2/tank

Tank bottom: Sand bottom over concrete

Breeding water temperature: 20.2 to 27.1°C

Water depth: Approx. 1 m

Water volume: Water circulated by water wheel with hardly
any change of water

Feed: Shrimp larvae dry pellets no. 2, Kyowa Hakko Kogyo

Amount of microorganisms added: As shown in Table 5

The feed in tanks 1 and 2 was prepared by adding microorganisms at 0.1% of the weight of the feed and dissolving in water equal to 10% of the weight of the feed followed by uniformly spraying onto the feed.

[0028]

Table 5

	Tank 1	Tank 2	Tank 3	Tank 4
Lactobacillus SHO-31	4×10^{10}	4×10^{10}	-	-
Bacillus subtilis natto	-	2×10^{10}	-	-
Yeast	-	1×10^{10}	-	-
Initial total number of prawns	390,000	390,000	390,000	390,000
Total number of prawns after 38 days	260,042	256,016	214,528	224,319
Weight of prawns produced after 38 days (kg)	121.7	123.4	113.7	115.3
Yield (%)	66.7	65.64	55.00	57.51
Total amount of feed given (kg)	153	153	153	153
Total weight gain (kg)	118.5	120.1	110.5	112.1
Feeding efficiency (%)	77.5	78.5	72.2	73.3
Weight gain coefficient	1.29	1.27	1.38	1.36

[0029]

[Effect of the Invention]

According to the culturing method of the present invention, in addition to preventing diseases of cultured fish and shellfish and promoting the growth of fish and shellfish, the method is also superior with respect to safety as well.

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